REMARKS

1. Status Information:

Claims 1, 2-44 and 46-48 are under consideration. Claim 1 is hereby amended.

2. Amendments to Claim 1:

Claim 1 has been amended to eliminate the issues supporting the rejections under 35 USC 112, first and second paragraphs. The asserted unsupported and indefinite limitations *"essentially every"* (member) and *"identical vector backbone sequence"* have been deleted. Steps (a) and (b) have been re-written to avoid indefiniteness or new matter issues. Literal support for the limitations in amended steps (a) and (b) may be found in the Summary of the Invention section of the specification, page 4, line 29 through page 5, line 1, which text is reproduced verbatim below for the Examiner's convenience.

> *The nucleic acid molecules preferably comprise a nucleic acid sequence that is identical for each molecule (member of the library) and that includes an origin of replication (and optionally other regulatory sequences, and/or vector backbone, etc.) ; and a nucleic acid sequence that varies between members of the population and comprises at least one substrate for recombination.*

3. Rejections Under 35 USC 112, 2nd:

Claims 1, 2, 4-44 and 46-48 were rejected under 35 USC 112, second paragraph, as containing the indefinite limitations *"essentially every member" and "identical vector backbone sequence"*. Claim 1 has been amended to remove

these limitations, thereby obviating the specific rejections. Amended claim 1 recites clear, unambiguous language taken directly from the specification as filed. One of skill in the art can easily determine the metes and bounds of the phrase *"a nucleic acid vector sequence"*. Similarly, the language of amended step (b) is definite, unambiguous and literally supported by the specification as filed.

4. Rejections Under 35 USC 112, 1st:

Claims 1, 2, 4-44 and 46-48 were also rejected under 35 USC 112, first paragraph, as failing to comply with the written description requirement, on the basis that the phrases *"essentially every member" and "identical vector backbone sequence"* are not supported by the specification and constitute new matter. As explained under paragraph 3, above, these phrases have been deleted, thereby obviating the rejections.

5. Rejections Under 35 USC 102(b) – Johnson et al.

The rejection of claims 1, 2 and 4-48 under 35 USC 102(b) as being anticipated by Johnson et al., 2001 were maintained.

Johnson et al. is directed exclusively to "two-vector" recombination systems, wherein typically a phage or phagemid vector is used in combination with a plasmid vector to achieve recombination – the combination involves two different vectors, each having a different origin of replication. The subject invention is directed to a "single vector" recombination system, wherein a single vector carries the variable sequences which are to be recombined. This is a fundamental difference.

More specifically, Johnson et al. requires the use of two different vectors. See, for example, Johnson et al. column 12, lines 39-41:

> *The first vectors may be phages or phagemids and the second vectors plasmids, or the first vectors may be plasmids and the second vectors phages or phagemids.*

That Johnson et al. is only directed to "two-vector" recombination is consistently made clear throughout its specification. See, further, column 10, lines 28-43 and column 12, line 54 through column 13, line 2; most notably, the disclosure from line 67 of column 12 through line 2 of column 13 (*"said population of said second polypeptide chains not being expressed from the same vector as said population of first polypeptide chains"*). In addition, see column 14, lines 52-53 (*"The genes for both subunits present on two separate replicons..."*), and column 17, lines 29-30 (*"the concept of using two or more replicons to generate diversity..."*).

Similarly, Fig 2 of Johnson et al. shows a recombination scheme using distinct phage and phagemid libraries. Fig. 3A shows a recombination scheme using distinct phagemid and plasmid libraries, wherein two different origins of replication are indicated.

There is no disclosure in Johnson directed to a "single-vector" approach to recombination.

As explained in the subject application (page 3, lines 12-30), the state of the prior art was essentially as follows:

> *The introduction of the different expression vectors carrying the genes to be recombined within the cell, however, posed serious problems to the creation of large diverse libraries. Typically, the two vectors had to be different (e.g. on e vector a plasmid, the other vector a phage). This is because it had been reported that bacteria*

> *infected by a filamentous phage are resistant to further infection by other filamentous phage (Boeke et al. (1982) Mol Gen Genet 186: 185-192). One of the two vectors thus had to be subject to transfection rather than infection and this drastically lowered the efficiency of transformation of the cell.*
>
> *In addition, it was believed that the two constructs required different origins of replication belonging to different incompatibility groups and also different antibiotic resistance genes. This is because it was believed by experts in the field that plasmids that utilize the same replication system (origin of replication) cannot co-exist stably in a cell are said to be incompatible (see pages 1.3-4 of (Sambrook et al. (1989) Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press)). For each plasmid and origin of replication, a different antibiotic resistance gene was also required. This limited the total number of different plasmids which could be maintained within a single cell. Consequently, the number of recombination substrates that could be present within a single cell was limited to the number of different origins of replication and antibiotic resistance genes that could be used. Thus, the size and diversity of a library that could be made using this approach was seriously limited.*

The subject invention specifically overcomes the various limitations of all two-vector approaches in the prior art, including the approach described by Johnson et al. The invention was, in part, the result of the unexpected discovery that recombination could occur between sequences located on the same type of vector. See Example 1 in the specification, beginning on page 41, as well as page 21, lines 1-8, reproduced in part below:

> *It was surprising discovery of this invention that, contrary to the teachings in the prior art, multiple constructs (vectors) of the same class (phagemid, plasmid, etc.) that are essentially identical in regulatory elements (e.g., origin and/or promoters and/or enhancers and/or termination sequences) and/or selectable markers (e.g., antibiotic resistance genes) can co-exist in a host cell (e.g. bacterial cell) for a sufficiently long time that extensive recombination ... can occur...*

Before the invention, the prior art taught that vectors having different origins of replication were required in order to achieve recombination of sequences resident on two different vectors, and not between sequences resident on the same vector. The invention provided the art, for the first time, with the knowledge that vectors having the same origin of replication could facilitate recombination between sequences on different vectors.

In one embodiment described in the specification of the subject application (see FIG. 1), identical phagemids containing identical origins of replication are used to achieve recombination between variable heavy and variable light antibody chains. The experiments described in Examples 1 and 2 (beginning on page 41 of the specification) showed that at least five different phagemid could enter a single bacteria, and that two phagemid that entered a single bacteria were able to recombine with each other to equilibrium, resulting in four different phagemid. Example 3, beginning on page 46 of the specification, describes the successful use of identical phagemids to achieve extensive recombination of antibody variable heavy and light chain genes to generate a highly diverse scFv antibody library.

The limitation of step (a) of amended claim 1 is simply not disclosed in Johnson et al.

6. Conclusion:

Applicants have amended claim 1 for clarity, as required. In addition, the foregoing discussion explains the fundamental distinction between the claimed methods (single-vector approach) and the cited prior art (two-vector approach). With respect, the claims are now in condition for allowance. Reconsideration and withdrawal of all pending rejections is therefore kindly requested.

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Respectfully submitted,

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